Molecular Biology Research Communications 2014; 3 (Suppl. 1): 282 MBRC

Poster – P205 Open Access

The National Conference on Protein & Peptide Sciences: From Basic to Medical & Industrial Applications

Shiraz University 10 & 11 December 2014

Expression and purification of the recombinant kinase domain of FGFR2b and study of its structural changes due to the interaction with gallic acid

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ABSTRACT

FGFR2b plays a significant role in cell signaling pathway, regulating several key biological processes including cellular differentiation and proliferation. Genetic alterations of the tyrosine kinase domain of FGFR2b occur in many tumor cells. Several epidemiological and animal studies have demonstrated that flavonoids such as gallic acid (GA) can reduce the growth and proliferation of many different human tumor types.The aim of this study was to express and purify the recombinant human FGFR2b kinase domain and analysis of its structural changes upon interaction with GA. Recombinant pLEICS-01 vectors containing the target gene were transformed into E coli BL21. Expression of recombinant protein was analyzed by SDS-PAGE. The protein was purified by affinity chromatography and the protein sample was dialyzed and then used to assess its interaction with both wild type and mutant SH2 domains of PLC, using PAGE. Chemical denaturation and intrinsic fluorescence spectra of the purified proteins were carried out by adding different concentrations of GA. Results, using the PAGE approach, confirmed that the purified protein was in active state. The intrinsic fluorescence assessment of kinase domain in the presence of gallic acid shows an increase in the intensity and maximum emission wavelength. According to the obtained results, the recombinant kinase domain of FGFR2b (38 kDa) was expressed, solubilized, purified and confirmed that it was in active state. The tertiary structural change of kinase domain reflects a conformational alteration within the protein that is important for the biological function of FGFR2b.

Key words: Fibroblast growth factor receptor; Protein purification; Kinase domain; Gallic acid; Fluorescence spectroscopy